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IDEA Grant: Effects of Tobacco Smoking in Clear Cell Renal Cell Carcinoma

PI: Maria F. Czyzyk-Krzeska, M.D., Ph.D.

Progress Report Year 1

Introduction:

Clear cell renal cell carcinoma (ccRCC) is the most common malignant form of renal cancer that occurs more frequently in males than in females¹. Tobacco smoking (TS), obesity, hypertension, and age are established risk factors for ccRCC development¹. Despite the well published and strongly documented association between TS and ccRCC, the genetic and signaling landscape of effects of TS on ccRCC is very little understood. The Cancer Genome Atlas (TCGA) database and the seminal report from The Cancer Genome Atlas Research Network, published in July 2013 in Nature, established the genetic landscape of ccRCC but did not focus on the relationship of ccRCC with smoking. This is a gap in our understanding of this important risk factor and genetic cause of ccRCC. Here we hypothesize that TS-derived oncogenic metabolites cause distinctive genomic and signaling changes in kidney/tumor tissues and promote ccRCC tumor growth. Because of a high prevalence of smoking in active military personnel and veterans, the male military population over the age of 40 years has an overall 5- to 6-fold increased incidence of ccRCC compared to the general population (Defense Medical Epidemiology Database, DMED, 1995-2004). The main purpose of the work is to collect and genetically analyze primary ccRCC tumors from a cohort of male veteran patients who are heavy smokers.

Keywords: renal cancer, clear cell renal cell carcinoma, tobacco smoking, von Hippel Lindau tumor suppressor

Accomplishments:

What were the major goals of the project?

It is a discovery level study to collect and genetically analyze primary ccRCC tumors from a cohort of male veteran patients who are heavy smokers and also those who are non-smokers.

What was accomplished under these goals?

The performed work is focused on Task 1 and Task 6, i.e. collection of tumor/kidney specimens from VA patients smokers and non-smokers and on building the data base of information characterizing the tissues and the patients. The very initial task was to organize an efficient pipeline for identifying and consenting the patients, as well as for collecting the fresh-frozen and formalin-fixed tissues from the surgical pathology.

This pipeline was finalized by February 2015. The delay was caused by the fact that surgeon, Dr. Gaitonde was out of country because of a family emergency and because it took us time to identify the most appropriate study coordinator and laboratory research assistant (the previous person has left the job). At this point, we have well organized network of a study coordinator who directly interacts with the surgeon and patients, surgical pathologist who identifies histological types of tumors and actually flash-freezes the specimens, and a laboratory technician who processes the tissues and organizes the materials.

Since then, we have collected 10 sets of specimens from all performed surgeries. Table 1 and Table 2 provide specific information regarding the collected tumors. All patients are Caucasian males. Additional unlisted in the Tables information include number of packs of cigarettes smoked per day and number of years of smoking, as well as number of years or months since cessation of smoking. We have also begun Task 2, i.e. DNA and RNA extraction from tumors and kidneys and are in the process of sequencing VHL gene as well as other ccRCC tumor suppressors, PBRM1, BAP1, SETD2, p53.

What opportunities for training and professional development has the project provided? Nothing to Report

Table 1.
Baseline characteristics
of study patients

N	10
Age (years)	58± 2.4
BMI(kg/m ²)	28.8± 1.7
Hypertension	5
Smoker:	
current	7
former	1
never	2
Kidney Cancer Histopathology:	
clear cell	4
papillary	2
chromophobe	0
oncocytoma	1
others	3

Table 2.
Baseline characteristics of patients
with clear cell carcinoma

N	4
Age (years)	58± 3.4
BMI(kg/m ²)	29.9 ±1.6
Hypertension	3
Smoker:	
current	3
former	0
never	1
ccRCC:	
grade 1	0
grade 2	0
grade 3	3
grade 4	1
Normal kidney	3
Adjacent kidney	3
Nephrectomy:	
partial	3
total	1
average time from clamping (min)	52.8± 12.4

How were the results disseminated to communities of interest? Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

The construction of libraries and RNAseq and WES will begin when we get a minimum of 10 ccRCC tumors and kidneys. Simultaneous processing of a larger batch of tissues is necessary to minimize any potential technical errors.

Impact:

At this stage the work is in progress.

What is the impact on the development of the principal discipline of the project? Nothing to Report

What was the impact on other discipline? Nothing to Report

What was the impact on technology transfer? Nothing to Report

What was the impact on society beyond science and technology? Nothing to Report

Changes/Problems:

Changes in approach and reasons for change: Nothing to Report

Actual or anticipated problems or delays and actions of plans to resolve them:

The number of clear cell RCC collected is somewhat lower than anticipated. The prevalence of ccRCC in the total population is about 75-80%, thus we were expecting to have at least 7 out of 10 tumors to be ccRCC. However we have only 4. While this trend may be simply accidental, it is possible that the VA population has somewhat different distribution of incidence of different histological subpopulation of ccRCC as compared to the general population. If so, this observation may be important information. Still we shall be able to reach the minimum number of 10 ccRCCs from smoker VA patients to allow us the initial analysis. We plan to collect tumors from VA patients in order to build solid tumor bank even beyond the duration of the present grant.

In order to increase the number of tumors from both smokers and non-smokers, we have contacted National Disease Research Interchange (NDRI), an organization that procures different human specimens for research. The samples are collected according to the customized requests and deidentified. We have used this service in the past in the procurement of our collection of 128 human ccRCC and normal kidneys and obtained data that we have published in several papers. NDRI is able to procure tissues from patients with known status of smoking and additional information such as BMI and history of hypertension. The advantage of specimens from NDRI is that it will be easier to obtain tumors from non-smokers, since majority of VA patients are smokers. These specimens are deidentified. Thus we plan to begin collecting these tumors through NDRI. However, NDRI does not partner with VA hospitals, thus the tissues will not come from the VA patients.

As a result we will likely collect two complementary cohorts of tissues, one from VA smoker patients, and another from non-VA smoker and non-nonsmoker patients.

Changes that had significant impact on expenditures: Nothing to report

Significant changes in use of or care of human subjects, vertebrate animals or biohazards and select agents: Nothing to Report

Products:

Biospecimens collection: We have initiated collection of valuable tissues from VA patients with renal cancer. This forms a basis for building of a tissue bank for renal cancer.

Participants & Other Collaborating Organizations:

What Individuals have worked on the project?

Dr. Czyzyk-Krzeska, PI
Department of Cancer Biology, UC, VAMC: *No change*

Dr. J. Meller, co-Investigator
Department of Environmental Health, Division of Bioinformatics, UC: *No change*
Dr M. Medvedovic, co-Investigator

Department of Environmental Health, Division of Bioinformatics, UC: *No change*
1 cal. month

Dr. Gaitonde, site-Investigator
Chief, Section of Urology, Cincinnati, VAMC: *No change*
1 cal. month

Dr. Thakar, co-Investigator
Chief, Renal Section, Cincinnati, VAMC: *No change*

Dr. Bui, collaborator
Staff Pathologist, VAMC: *No change*

Stephanie Ross, RN
Study Coordinator, VAMC
Ms Ross replaced the original coordinator, Ms Burke: 2 cal. months

John Chu, BS
Research Assistant, UC
Mr. Chu replaced Mr Abplanalp: 6 cal. months

Has there been a change in the active other support of the PD/PI or senior key personnel? No

What other organizations were involved as partners?

Cincinnati VA Medical Center
Research Service,
3200 Vine Street,
Cincinnati, OH 45069-2213

VA site: collection of all specimens as well as clinical, histopathological and general information about the patients and tissues.

Special Reporting Requirements: Nothing to Report

Appendices: Nothing to Report